PRELIMINARY REMARKS

Applicants thank the Examiner for the telephone interview conducted October 20, 2005. Applicants also note an apparent typographical error in the Advisory Action mailed November 8, 2005. Item 1.a) states that the period for reply expires "*5* months" from the mailing date of the final rejection. Because the final rejection was mailed June 6, 2005, the stated period for reply would expire November 6, 2005, which is two days before the mailing of the Advisory Action. Applicants believe that "*6* months" may have been intended. However, if Applicants are in error as to this belief, then clarification is respectfully requested.

REMARKS

Claims 1-48 are now pending in the application, of which Claims 23-26 and 37-44 are withdrawn, and Claims 1-22, 27-36 and 45-48 are rejected. Claims 1-4, 8-9, 11, 13-14, 18-19, 23-26, 45-48 have now been amended. Page 8 of the Specification has now been amended and a Sequence Listing has now been added following page 28 of the Specification.

The Specification has now been amended to insert two paragraphs at page 8 and to insert a Sequence Listing (14 pages) following page 28. These are taken from copending US Serial No. 10/220,986, which has already been incorporated by reference in the present Application: see page 7, lines 11-15 of the present Application. In particular, the two paragraphs inserted at page 8 are copied from paras. [0108] and [0118] of the publication of copending US Serial No. 10/220,986, i.e. US Publication No. 2004/077067 A1 of Sin et al. for

Therapeutic and prophylactic agents derived from Aeromonas hydrophila *bacterial surface proteins* (published April 22, 2004).

The 14-page Sequence Listing comprises the Sequence Listing of the already-incorporated '986 application, with minor modifications: 1) to meet the requirements of PatentIn 3.3 by a) eliminating termination codons from the numbering of the recited coding sequences, and b) adding recitation of the present Application data and Applicant information; and 2) to include a recitation of the publication for SEQ ID NO:9 as described in the '986 application (see, e.g., paras. [0008] and [0295], and Fig. 1 (panel A), of the '067 publication). It is respectfully submitted that no new matter is presented by the present amendments to the Specification.

Minor amendments have been made to the claims to simply overcome rejections of the claims under 35 U.S.C. § 112 and to further clarify the subject matter thereof without narrowing the scope. In particular: Claims 2, 21, and 45 have now been amended to add an indefinite article; Claims 3, 4, 8, 9, 13, 14, 18, 19, and 24-26 have now been amended to remove the extraneous term "further;" Claims 21 and 45 have now been amended to correct an inadvertent typographical error that had resulted in recitation of "viruses elected from" rather than the obviously correct phrase "virus selected from" the defined group; Claims 22-23 and 46-48 have now been amended to correct or update punctuation (commas and semicolons); and Claim 23 has now been amended to correct typographical errors in systematic bacterial names. Applicants submit that these are not narrowing amendments.

Likewise, Claims 11, 23, and 48 have now been amended to better clarify that the "FP" protein is recombinant protein comprising "immobilization antigen repeat I" of *Ichthyophthirius multifiliis*. Support for this amendment is found, e.g., at page 3, lines 24-25, page 8, lines 18-27, and in Example II, of the Specification. Also, Claims 23 and 48 have now been amended to remove the phrase "whole recombinant protein AHMA" as a term redundant with the remaining recitations of the AHMA protein(s) and variants; and to replace the term "singly" with the clearer term "alone" (which is used, e.g., at page 8, lines 5-7 of the Specification). Applicants point out that these are not narrowing amendments.

Similarly, Claims 23 and 48 have now been amended to expressly recite that the "oral vaccine" defined by the claim as an emulsion, paste, or particulate, is "orally suitable"; and Claim 1 has now been amended to recite that the oral vaccine defined thereby is provided in an "orally suitable" formulation. Support for these amendments is found: throughout Specification, which consistently describes the claimed vaccines as "oral;" and, e.g., at page 9, line 32 to page 10, lines 34 of the Specification (paras. [0044]-[0048] of US Publication 2005/0118194 A1), which describes the ingredients that are "suitable" for use in an oral vaccine composition hereof in terms of "biologically beneficial" or "biologically inert" ingredients that are, e.g., "edible" materials, "feed," "non-toxic" oils, "metabolizable" components, and so forth. Because oral suitability is a feature of all "oral vaccine" compositions that are actually useful as "oral vaccines," Applicants submit that these amendments are likewise not narrowing amendments.

Claims 1, 23, and 48 have now been amended: (1) to recite that the recombinant AHMA protein has the amino acid sequence of any one of SEQ ID NOs:2, 4, or 8, with SEQ ID NOs:2 and 4 referring to the now Sequence-Listed AHMA preprotein, and SEQ ID NO:8 referring to the now Sequence-Listed AHMA mature protein; (2) to recite that this recombinant AHMA protein is an "isolated" recombinant AHMA protein; (3) to better define the protein fragments useful in an oral vaccine as "immunogenic" fragments of the recombinant AHMA protein; and (4) to better define the recited recombinant protein derivatives as "immunogenic conservative amino acid-substituted variants" of the AHMA protein that are "at least 75% homologous thereto", or "immunogenic" fragments of those variants. Support for these amendments to Claim 1 is found, e.g.: in the two paragraphs now inserted at page 8 (for the "SEQ ID NOs:2, 4, or 8," "conservative amino acid substituted variant," and "at least 75% homologous" elements); at page 6, lines 9-12, and page 13, Example 1 (for the "isolated" element); and at page 11, lines 13-21 (for the "immunogenic," and "conservative amino acid substituted variant" elements).

Finally, Claims 1, 23, and 48 have now been amended to recite that the vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*. Support for this amendment is found, e.g., at page 10, lines 26-30, and page 11, lines 1-11 and 29-31 of the Specification.

The Examiner is respectfully requested to reconsider and withdraw the rejection(s) in view of the amendments and remarks contained herein.

VARIOUS MATTERS

Items 1-4. Applicants thank the Examiner for considering the amendments and remarks submitted in the Response filed March 17, 2005, for considering Applicants' arguments in regard to the restriction requirement, and for withdrawing the noted objections to the Specification and Claims and the noted 35 U.S.C. § 112 rejections.

REJECTION UNDER 35 U.S.C. § 112

Item 5. Claims 1-22, 27-36 and 45-48 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly not being enabled by the Specification. The rejection alleges that the Specification does not reasonably provide enablement for *derivatives* of the recombinant protein major adhesin protein of *Aeromonas hydrophila* (AHMA).

Both of the pending independent claims, Claims 1 and 48, have now been amended to recite: that the AHMA protein is an isolated protein having the amino acid sequence of any one of SEQ ID NOs:2, 4, or 8; that the derivatives are conservative substituted versions thereof that are at least 75% homologous thereto; and that the derivatives and fragments are immunogenic. The sequences and homology limitations have been imported from copending US Patent Application No. 10/220,986 to Sin et al. (the '986 application), which has already been incorporated by reference. Description of conservative amino acid substitutions has also been imported into the present Specification from the '986 application.

Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

REJECTION UNDER 35 U.S.C. § 102

Item 6. Claims 1, 27-29, 35-36, and 48 stand rejected under 35 U.S.C. § 102(a) as allegedly anticipated by Irianto et al. (*Journal of Fish Diseases*, February 2003, 26, 117-120). This rejection is respectfully traversed.

Irianto et al. discuss oral administration of formalin-inactivated cells of *Aeromonas hydrophila* A3-51 to treat *A. salmonicida* infection in goldfish. The oral vaccine composition in Irianto et al. comprises dead whole cells of A3-51 (see for example, page 118). Irianto et al. do not teach an oral vaccine composition comprising *isolated* recombinant protein AHMA, as defined by the presently amended Claims. Therefore Irianto et al. do not disclose even a single limitation found in claim 1. A prior art reference anticipates a claim only if it discloses *each and every* limitation found in the claim. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

Item 7. Claims 1-3, 5-6, 10, 27-29, and 48 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Fang et al. (*Journal of Fish Diseases*, 2000, 23, 137-145). This rejection is respectfully traversed.

Fang et al. disclose *intraperitoneal* immunization of blue gourami with the *Aeromonas hydrophila* major adhesin protein (a 43 kDa Outer Membrane Protein) in the presence of Freund's complete adjuvant (FCA). In animals,

contact with Freund's complete adjuvant is reported as causing inflammation, induration, necrosis, hyperalgesia, chronic granulomas, ulcerations, abscesses, tissue sloughs, arthritis, neural or mechanical lameness, and peritonitis. As a result, ethical use of FCA in animals is generally limited to non-oral use in those situations where no more humane substitute adjuvant is available. By choosing the very powerful FCA as the adjuvant in their parenteral vaccine composition, Fang et al. implicitly suggest that the composition thereof is not suitable for oral administration.

Further, Fang et al. do not address whether or not a vaccine composition containing proteins that exhibit immunogenicity when parenterally placed directly into, e.g., intramuscular, intravascular, or intraperitoneal, tissue can be administered orally to fish so as to retain significant immunogenicity. It is well known that the route of delivering a vaccine is an important factor for successful immunization (see for example, page 3, 1st sentence of the Specification) and can influence the strength of the resulting immune response. Also, it is commonly understood that the vertebrate digestive system is effective to denature and hydrolyze many proteins. In this light, Fang et al. does not contain any teaching to provide AHMA proteins or their immunogenic fragments or derivatives in an orally administrable composition. Likewise, Fang et al. do not suggest oral administration of their FCA-containing composition.

In contrast to Fang et al., both of the pending independent claims, Claims 1 and 48, have now been amended to recite, in the body of the claim, that the vaccine composition is an "orally suitable" formulation of AHMA protein(s) having recited sequence(s), or their immunogenic variant(s) or fragment(s); and that the

vaccine composition is effective to elicit immunization when orally administered. As noted above, Fang et al. do not disclose oral administration of an AHMA vaccine nor that their AHMA composition would be suitable for oral administration, nor that it would effectively function as a vaccine even if it were administered orally.

Likewise, Fang et al. fail to describe isolated *recombinant* protein AHMA in general, and more specifically fail to describe the recited AHMA sequences, variants, and fragments as presently claimed. Therefore, since Fang et al. do not disclose *each and every* limitation found in Claims 1 and 48, it is respectfully submitted that Fang et al. do not anticipate the presently rejected Claims. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

REJECTION UNDER 35 U.S.C. § 103

Item 8. Claims 1-3, 5-6, 10-13, 15-16, 20-21, 27-36, 45, and 48 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et al. (U.S. Pat. No. 5,284,653 published February 8, 1994) in view of Wang et al. (*Fish Shellfish Immunol.*, Nov. 2002; 13(5):337-50). This rejection is respectfully traversed.

Wolf-Watz et al. describe a fish vaccine comprising whole-cell (preferably live) avirulent, invasive and immunogenic strain of a fish pathogenic bacterial species such as *Aeromonas hydrophila*. Wang et al. describe a vaccine composition comprising the surface immobilization antigen of the protozoan, *Ichthyophthirius multifiliis*, and Freund's complete adjuvant (FCA).

The rejected claims, as presently amended, define a vaccine composition comprising at least one of isolated recombinant protein AHMA having a specified amino acid sequence, or defined derivatives or fragments, optionally in combination with another membrane protein and/or inactivated bacterial strains and/or inactivated viral strains, the composition being orally suitable and effective, upon oral administration, to elicit immunization against *Aeromonas hydrophila*.

In light of the claims as presently amended, even if there were motivation to combine Wolf-Watz et al. with Wang et al., this would not have made the present invention obvious to one of ordinary skill in the art. An *Aeromonas*-type whole-cell vaccine of Wolf-Watz et al. utilizes whole, preferably live, *Aeromonas* cells. Neither of the cited references describes the AHMA proteins as presently claimed, and neither describes the use of isolated recombinant AHMA proteins in a vaccine, much less in an oral vaccine. Moreover, Wolf-Watz et al. teach away from the use of isolated AHMA proteins in that these authors state that vaccines based on single "bacterial components" confer an inferior degree of immunity, an inferior duration of immunity, and a less complete immunization, while requiring more processing steps, possibly greater expense, and often a greater dosage than whole-cell (live) vaccines (see, for example, '653 column 2, lines 27-41).

In contrast to the Wolf-Watz et al. negative characterization of the effectiveness of purified protein vaccines, the present inventors have found that vaccine compositions comprising isolated, recombinant AHMA protein provides an effective vaccination against *Aeromonas hydrophila* infection, including when orally administered. See Examples VI and VII at pages 16-18 of the

Specification, with Table 1 thereof and Figure 1, showing significantly increased survival rates against *Aeromonas hydrophila* infection by fish immunized with a vaccine composition comprising isolated, recombinant AHMA protein, either as the sole bacterial antigen or in combination with other bacterial/protozoal/viral antigens. As a result, in light of the Wolf-Watz et al. statements, one of ordinary skill in the art would have found the present invention surprising and unexpected.

Moreover, the present invention also involved: selecting one particular type of antigenic protein, AHMA protein(s), out of all the types of antigenic proteins presented on the surface of *Aeromonas hydrophila*. In contrast to whole cells, against which the immune system can raise an immune response to all such surface-presented antigens to thereby confer effective immunity against the organism, selection of a single antigen does not provide a basis for one of ordinary skill in the art to reasonably expect that it could confer effective immunity against the same whole organism. The fact that a protein is immunogenic does not address whether or not it could provide immunity against the whole organism from which it is derived, so as to be effective as a basis for a vaccine composition (i.e. a vaccine composition that functions as a vaccine composition). In the particular case of *Aeromonas hydrophila*, the contrast of a single protein versus whole cells is even more pronounced, since this organism presents a great diversity of surface-presented antigens: "...the antigenic diversity of *A. hydrophila* has posed a great difficulty to vaccine development..." (see p. 137. col. 2 of Fang et al. discussed above under Item 7).

In addition, one of ordinary skill in the art would not have found it obvious that an oral route of administration for an isolated recombinant AHMA protein

composition could result in effective immunity. In contrast to administration of whole cells, administration of isolated proteins presents additional uncertainties in achieving effective immunization. Exemplary differences are described as follows. Administering a vaccine composition comprising whole cells as the active agent provides a number of advantageous features for the immunization process, which include: that the whole cell provides a self-buffering capability against digestive tract pH extremes, and provides additional compounds and structures that can mitigate the protein-denaturing effect of the digestive tract environment, which thereby can help maintain protein immunogenicity and optimal presentation orientation; and, in the case of living cells, that the cell continues to synthesize additional immunogenic proteins, and in some cases may be robust enough to pass through the stomach and into the intestine, where it can continue undergoing cell growth and division to provide still further immunogenic proteins before being passed with waste.

In addition to these general properties of whole cells, *Aeromonas* species are Gram(-), gamma-proteobacteria that provide additional self-protective features in that such organisms have both an inner and an outer membrane, defining a periplasmic space, with the outer membrane comprising a protective, phospholipid-peptidoglycan-lipopolysaccharide triple layer. These features can permit preservation of immunogenicity of surface-presented proteins and can maintain immunogenicity by synthesis of new surface-presented immunogenic proteins. Presentation of the immunogenic proteins by the cell surface also permits preferential orientation of each of the antigenic proteins vis-à-vis the immune system, in the same orientation as the immune cells will contact them in

later (challenge) infections. This can further enhance the odds that a whole cell will raise an immune response that will be targeted to, and will specifically recognize, the infectious organism as encountered in future.

In contrast, oral presentation of isolated bacterial proteins normally results in degradation of the protein as food, with passage into waste of unused protein. Use of whole recombinant proteins (whether in mature or pre-protein form), even where an immune response is elicited, provides additional immunogenic targets each of which is an additional, and in some cases may be the sole, point of recognition by the immune system, thereby altering the chances of achieving immunization against the whole organism as it will be encountered.

As a result, in the specific case of *Aeromonas*, an isolated immunogenic *Aeromonas* protein, such as is recited in the present claims, would have been reasonably expected to face a *much* greater likelihood of failing to raise an infection-protective immune response than would the same proteins administered as part of the *Aeromonas* whole cell. As a result of these factors, and in light of the negative teaching of Wolf-Watz et al., one of ordinary skill in the art would have had no basis on which to form a reasonable expectation that oral administration of an isolated recombinant AHMA protein composition could result in effective immunization against *Aeromonas hydrophila* infection.

In regard to Wang et al., this reference does not provide what is lacking from Wolf-Watz et al., nor does its description of an isolated protozoal protein vaccine composition counter or overcome Wolf-Watz' et al. teaching away from the use of isolated bacterial proteins as vaccine composition actives. Consequently, one of ordinary skill in the art would not have found obvious, and

instead would have found motivation to avoid even attempting to obtain, oral vaccine compositions as defined by the amended claims, based on knowledge of the cited references.

Applicants also point out that, just as the term "recombinant," the term "isolated" of the present claims is a substantive limitation on the recombinant AHMA protein recited therein. Regardless of whether or not it is also considered a process limitation, the term "isolated," just as the term "recombinant," substantively distinguishes the recited AHMA protein from AHMA proteins existing in the natural state. In the case of the term "isolated," the distinction is from those AHMA proteins that exist in situ in bacterial cells, e.g., such as cells referred to by Wolf-Watz et al. Likewise, the presently amended Claims' recitation that the compositions are in the form of "orally suitable" compositions, is also a substantive limitation on the claimed compositions, as is the limitation recitation that the vaccine compositions are capable, when orally administered in an immunologically sufficient amount, of effecting immunization against *Aeromonas hydrophila*.

As a result, Applicants submit that the combination of the cited references does not provide the elements of the invention as presently claimed and would not have made the claimed invention obvious to one of ordinary skill in the art. Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be withdrawn.

Item 9. Claims 1-3, 5-6, 10-13, 15-16, 20-22, 27-36, and 45-48 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et

al., Wang et al. as set forth in Item 8 above, and further in view of Morinigo et al. (*Bulletin of the European Association of Fish Pathologists*, Nov. 2, 2002, Vol. 22, No. 5, pp. 298-303). This rejection is respectfully traversed.

The teachings of Wolf-Watz et al. and Wang et al. are described above under Item 8. Morinigo et al. discuss a divalent vaccine composition comprising formalized (inactivated) whole cells and extracellular products (ECPs) of virulent strains of *Vibrio alginolyticus* and *Photobacterium damselae*. Yet, Morinigo et al. do not provide what is lacking from the combination of Wolf-Watz et al. and Wang et al.

As noted above, Wolf-Watz et al. teach away from the invention as presently claimed, and the combination of Wolf-Watz et al. with Wang et al. does not provide one of ordinary skill in the art with a reasonable basis for obtaining an oral vaccine composition comprising isolated recombinant AHMA protein as an immunogenic active ingredient as presently claimed, i.e. a composition that actually is an oral vaccine against *Aeromonas hydrophila* and/or other pathogenic bacteria. Therefore, even if there were motivation to combine Wolf-Watz et al. with Wang et al., and to further combine Morinigo et al. therewith, the resulting combination would not have made the present invention obvious to one of ordinary skill in the art.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be withdrawn.

Item 10. Claims 1-6, 10-16, 20-22, 27-36 and 45-48 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Wolf-Watz et al., Wang et al. and Morinigo

et al. as set forth in Items 8 and 9 above, and further in view of Chen et al. (U.S. Patent No. 6,720,001 B1, published April 13, 2004). This rejection is respectfully traversed.

Wolf-Watz et al., Wang et al., and Morinigo et al. are discussed above under Items 8 and 9. Chen et al. disclose pharmaceutical oil-in-water emulsions for delivery of polyfunctional active ingredients. Chen et al. teach that the oil component of the oil-in-water emulsion may not be appropriately polar to effectively incorporate polyfunctional active ingredients at desirable therapeutic levels, without compromising product safety (see, for example, column 2, lines 7-12). In order to overcome this problem, Chen et al. disclose oil-in-water emulsions "wherein the oil phase includes components chosen to increase the polarity of the oil phase"... etc. (see column 3, lines 55-62). Yet, Chen et al. do not provide do not provide what is lacking from a combination of Wolf-Watz et al., Wang et al., and Morinigo et al.

As noted above, Wolf-Watz et al. teach away from the invention as presently claimed, and the combination of Wolf-Watz et al. with Wang et al. and Morinigo et al. does not provide one of ordinary skill in the art with a reasonable basis for obtaining an oral vaccine composition comprising isolated recombinant AHMA protein as an immunogenic active ingredient as presently claimed, i.e. a composition that actually is an oral vaccine against *Aeromonas hydrophila* and/or other pathogenic bacteria. Therefore, even if there were motivation to combine Wolf-Watz et al. with Wang et al. and Morinigo et al., and to further combine Chen et al. therewith, the resulting combination would not have made the present invention obvious to one of ordinary skill in the art.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be withdrawn.

Item 11. Claims 1-22, 27-36, and 45-48 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et al., Wang et al., Morinigo et al. and Chen et al. as set forth in Item 10 above, and further in view of Calanchi et al. (U.S. Patent No. 5,008,117, published April 16, 1991). This rejection is respectfully traversed.

Wolf-Watz et al., Wang et al., Morinigo et al., and Chen et al. are discussed above under Items 8, 9, and 10 above. Calanchi et al. teach a method of dispersing thickening agents in pharmaceutical formulations for effective delivery of micro-encapsulated drugs, which otherwise have the tendency to precipitate or float. Yet, Calanchi et al. do not provide do not provide what is lacking from a combination of Wolf-Watz et al., Wang et al., Morinigo et al., and Chen et al.

As noted above, Wolf-Watz et al. teach away from the invention as presently claimed, and the combination of Wolf-Watz et al. with Wang et al., Morinigo et al., and Chen et al. does not provide one of ordinary skill in the art with a reasonable basis for obtaining an oral vaccine composition comprising isolated recombinant AHMA protein as an immunogenic active ingredient as presently claimed, i.e. a composition that actually is an oral vaccine against *Aeromonas hydrophila* and/or other pathogenic bacteria. Therefore, even if there were motivation to combine Wolf-Watz et al. with Wang et al., Morinigo et al., and Chen et al., and to further combine Calanchi et al. therewith, the resulting

combination would not have made the present invention obvious to one of ordinary skill in the art.

Applicants believe that these remarks and amendments overcome the rejection and respectfully request that it be withdrawn.

CONCLUSION

It is believed that all of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider and withdraw all presently outstanding rejections. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (248) 641-1600. Favorable reconsideration and allowance of the Application is requested in light of the newly amended claims and accompanying remarks.

Respectfully submitted,

Dated: Dec. 5, 2005

By: _____

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PAK:MSS
- One paper form of the Sequence Listing (14 sheets)
- One computer readable form of the Sequence Listing on 3½ inch diskette
- One Statement of Identical Sequence Listings